

Amendments to the Claims:

1. (Currently amended) A method for detecting a mutant allele of a wheat acetohydroxyacid synthase large subunit (*AHASL*) gene that confers tolerance to imidazolinone herbicides on a wheat plant, said method comprising the steps of:

(a) obtaining genomic DNA from a wheat plant;

(b) using said DNA as a template for a PCR amplification comprising said DNA, polymerase, deoxyribonucleotide triphosphates, a forward *AHASL*-gene-specific primer, a reverse *AHASL*-gene-specific primer, and a mutant-allele-specific primer comprising a nucleotide sequence with a 5' end and a 3' end, wherein said nucleotide sequence is capable of annealing to the complement of nucleotides 3 to 23 of SEQ ID NO: 12, and wherein the 3'-end nucleotide of said nucleotide sequence is cytidine and said cytidine is at the position as illustrated in Figure 4 that corresponds to the site of the G-to-A point mutation that gives rise to the S653(At)N substitution in an *AHASL* protein; and

(c) detecting ~~the products~~ a product of said PCR amplification[[:]], said product corresponding to the region of said *AHASL* gene bounded by the annealing sites of the mutant-allele-specific primer and the reverse *AHASL*-gene-specific primer on said *AHASL* gene;

wherein said mutant-allele-specific primer is capable of annealing to a region of an *AHASL* gene that is nested between the annealing sites of said forward and reverse *AHASL*-gene-specific primers.

2. (Original) The method of claim 1, wherein said DNA has been subjected to a pre-amplification before step (b), said pre-amplification comprising said DNA, polymerase, deoxyribonucleotide triphosphates, a forward *AHASL* primer, and a reverse *AHASL* primer, wherein said forward and reverse *AHASL*-gene-specific primers are capable of annealing to regions of an *AHASL* gene that are nested between the annealing sites of said forward and reverse *AHASL* primers.

3. (Original) The method of claim 2, wherein said DNA is digested with exonuclease following said pre-amplification and before step (b).

4. (Original) The method of claim 2, wherein said forward *AHASL* primer and said reverse *AHASL* primer are designed to anneal to *AHASLIA*, *AHASLIB*, and *AHASLID*.

5. (Original) The method of claim 2, wherein said forward *AHASL* primer comprises the nucleotide sequence set forth in SEQ ID NO: 1.

6. (Currently amended) A method for detecting a mutant allele of a wheat acetohydroxyacid synthase large subunit (*AHASL*) gene that confers tolerance to imidazolinone herbicides on a wheat plant, said method comprising the steps of:

(a) obtaining genomic DNA from a wheat plant;

(b) using said DNA as a template for a PCR amplification comprising said DNA, polymerase, deoxyribonucleotide triphosphates, a forward *AHASL*-gene-specific primer, a reverse *AHASL*-gene-specific primer, and a mutant-allele-specific primer comprising a nucleotide sequence with a 5' end and a 3' end, wherein said nucleotide sequence is capable of annealing to the complement of nucleotides 3 to 23 of SEQ ID NO: 12, and wherein the 3'-end nucleotide of said nucleotide sequence is cytidine; and

(c) detecting ~~the products~~ a product of said PCR amplification[[:]], said product corresponding to the region of said *AHASL* gene bounded by the annealing sites of the mutant-allele-specific primer and the reverse *AHASL*-gene-specific primer on said *AHASL* gene;

wherein said mutant-allele-specific primer is capable of annealing to a region of an *AHASL* gene that is nested between the annealing sites of said forward and reverse *AHASL*-gene-specific primers, wherein said DNA has been subjected to a pre-amplification before step (b), said pre-amplification comprising said DNA, polymerase, deoxyribonucleotide triphosphates, a forward *AHASL* primer, and a reverse *AHASL* primer, wherein said forward and reverse *AHASL*-gene-specific primers are capable of annealing to regions of an *AHASL* gene that are nested between the annealing sites of said forward and reverse *AHASL* primers, and wherein said

reverse *AHASL* primer comprises the nucleotide sequence set forth in SEQ ID NO: 2.

7. (Original) The method of claim 1, wherein said mutant-allele-specific primer comprises the nucleotide sequence set forth in SEQ ID NO: 3.

8. (Original) The method of claim 1, wherein said *AHASL* gene is *AHASL1D*.

9. (Currently amended) A method for detecting a mutant allele of a wheat acetohydroxyacid synthase large subunit (*AHASL*) gene that confers tolerance to imidazolinone herbicides on a wheat plant, said method comprising the steps of:

(a) obtaining genomic DNA from a wheat plant;

(b) using said DNA as a template for a PCR amplification comprising said DNA, polymerase, deoxyribonucleotide triphosphates, a forward *AHASL*-gene-specific primer, a reverse *AHASL*-gene-specific primer, and a mutant-allele-specific primer comprising a nucleotide sequence with a 5' end and a 3' end, wherein said nucleotide sequence is capable of annealing to the complement of nucleotides 3 to 23 of SEQ ID NO: 12, and wherein the 3'-end nucleotide of said nucleotide sequence is cytidine; and

(c) detecting ~~the products~~ a product of said PCR amplification[[:]], said product corresponding to the region of said *AHASL* gene bounded by the annealing sites of the mutant-allele-specific primer and the reverse *AHASL*-gene-specific primer on said *AHASL* gene;

wherein said mutant-allele-specific primer is capable of annealing to a region of an *AHASL* gene that is nested between the annealing sites of said forward and reverse *AHASL*-gene-specific primers, wherein said *AHASL* gene is *AHASL1D*, and wherein said forward *AHASL*-gene-specific primer has the sequence set forth in SEQ ID NO: 5 and said reverse *AHASL*-gene-specific primer has the sequence set forth in SEQ ID NO: 7.

Claims 10-13. (Cancelled)

14. (Original) The method of claim 1, wherein said detecting comprises gel electrophoresis and ethidium-bromide staining.

15. (Currently amended) A method for analysis of a wheat *AHASL* gene, said method comprising the steps of:

(a) obtaining genomic DNA from a wheat plant;

(b) using said DNA as a template for a first PCR amplification comprising said DNA, polymerase, deoxyribonucleotide triphosphates, a forward *AHASL*-gene-specific primer, a reverse *AHASL*-gene-specific primer, and a mutant-allele-specific primer comprising a first nucleotide sequence with a 5' end and a 3' end, wherein said first nucleotide sequence is capable of annealing to the complement of nucleotides 3 to 23 of SEQ ID NO: 12, and wherein the 3'-end nucleotide of said first nucleotide sequence is cytidine and said cytidine is at the position as illustrated in Figure 4 that corresponds to the site of the G-to-A point mutation that gives rise to the S653(At)N substitution in an *AHASL* protein; and

(c) using said DNA as a template for a second PCR amplification comprising said DNA, polymerase, deoxyribonucleotide triphosphates, said forward *AHASL*-gene-specific primer, said reverse *AHASL*-gene-specific primer, and a wild-type-allele-specific primer comprising a second nucleotide sequence with a 5' end and a 3' end, wherein said second nucleotide sequence is capable of annealing to the complement of nucleotides 4 to 23 of SEQ ID NO: 10, and wherein the 3'-end nucleotide of said second nucleotide sequence is guanosine and said guanosine is at the position as illustrated in Figure 4 that corresponds to the site of the G-to-A point mutation that gives rise to the S653(At)N substitution in an *AHASL* protein; and

(d) detecting the products of said first and said second PCR amplifications; wherein said wild-type-allele-specific primer and said mutant-allele-specific primer are capable of annealing to a region of an *AHASL* gene that is nested between the annealing sites of said forward and reverse *AHASL*-gene-specific primers.

16. (Original) The method of claim 15, wherein said DNA has been subjected to a pre-amplification before step (b), said pre-amplification comprising said DNA, polymerase,

deoxyribonucleotide triphosphates, a forward *AHASL* primer, and a reverse *AHASL* primer, wherein said forward and reverse *AHASL*-gene-specific primers are capable of annealing to regions of an *AHASL* gene that are nested between the annealing sites of said forward and reverse *AHASL* primers.

17. (Original) The method of claim 16, wherein said DNA is digested with exonuclease following said pre-amplification and before step (b).

18. (Original) The method of claim 16, wherein said forward *AHASL* primer and said reverse *AHASL* primer are designed to anneal to *AHASLIA*, *AHASLIB*, and *AHASLID*.

19. (Original) The method of claim 16, wherein said forward *AHASL* primer comprises the nucleotide sequence set forth in SEQ ID NO: 1.

20. (Previously presented) A method for analysis of a wheat *AHASL* gene, said method comprising the steps of:

(a) obtaining genomic DNA from a wheat plant;

(b) using said DNA as a template for a first PCR amplification comprising said DNA, polymerase, deoxyribonucleotide triphosphates, a forward *AHASL*-gene-specific primer, a reverse *AHASL*-gene-specific primer, and a mutant-allele-specific primer comprising a first nucleotide sequence with a 5' end and a 3' end, wherein said first nucleotide sequence is capable of annealing to the complement of nucleotides 3 to 23 of SEQ ID NO: 12, and wherein the 3'-end nucleotide of said first nucleotide sequence is cytidine;

(c) using said DNA as a template for a second PCR amplification comprising said DNA, polymerase, deoxyribonucleotide triphosphates, said forward *AHASL*-gene-specific primer, said reverse *AHASL*-gene-specific primer, and a wild-type-allele-specific primer comprising a second nucleotide sequence with a 5' end and a 3' end, wherein said second nucleotide sequence is capable of annealing to the complement of nucleotides 4 to 23 of SEQ ID NO: 10, and wherein the 3'-end nucleotide of said second nucleotide sequence is guanosine; and

(d) detecting the products of said first and said second PCR amplifications; wherein said wild-type-allele-specific primer and said mutant-allele-specific primer are capable of annealing to a region of an *AHASL* gene that is nested between the annealing sites of said forward and reverse *AHASL*-gene-specific primers, wherein said DNA has been subjected to a pre-amplification before step (b), said pre-amplification comprising said DNA, polymerase, deoxyribonucleotide triphosphates, a forward *AHASL* primer, and a reverse *AHASL* primer, wherein said forward and reverse *AHASL*-gene-specific primers are capable of annealing to regions of an *AHASL* gene that are nested between the annealing sites of said forward and reverse *AHASL* primers, and wherein said reverse *AHASL* primer comprises the nucleotide sequence set forth in SEQ ID NO: 2.

21. (Currently amended) ~~The method of claim 15;~~ A method for analysis of a wheat *AHASL* gene, said method comprising the steps of:

(a) obtaining genomic DNA from a wheat plant;
(b) using said DNA as a template for a first PCR amplification comprising said DNA, polymerase, deoxyribonucleotide triphosphates, a forward *AHASL*-gene-specific primer, a reverse *AHASL*-gene-specific primer, and a mutant-allele-specific primer comprising a first nucleotide sequence with a 5' end and a 3' end, wherein said first nucleotide sequence is capable of annealing to the complement of nucleotides 3 to 23 of SEQ ID NO: 12, wherein the 3'-end nucleotide of said first nucleotide sequence is cytidine and said cytidine is at the position as illustrated in Figure 4 that corresponds to the site of the G-to-A point mutation that gives rise to the S653(At)N substitution in an *AHASL* protein, and wherein said mutant-allele-specific primer comprises the nucleotide sequence set forth in SEQ ID NO: 3[.];

(c) using said DNA as a template for a second PCR amplification comprising said DNA, polymerase, deoxyribonucleotide triphosphates, said forward *AHASL*-gene-specific primer, said reverse *AHASL*-gene-specific primer, and a wild-type-allele-specific primer comprising a second nucleotide sequence with a 5' end and a 3' end, wherein said second nucleotide sequence is capable of annealing to the complement of nucleotides 4 to 23 of SEQ ID NO: 10, and wherein the 3'-end nucleotide of said second nucleotide sequence is guanosine and

said guanosine is at the position as illustrated in Figure 4 that corresponds to the site of the G-to-A point mutation that gives rise to the S653(At)N substitution in an AHASL protein; and

(d) detecting the products of said first and said second PCR amplifications;
wherein said wild-type-allele-specific primer and said mutant-allele-specific primer are
capable of annealing to a region of an AHASL gene that is nested between the annealing sites of
said forward and reverse AHASL-gene-specific primers.

22. (Currently amended) ~~The method of claim 15;~~ A method for analysis of a wheat
AHASL gene, said method comprising the steps of:

(a) obtaining genomic DNA from a wheat plant;
(b) using said DNA as a template for a first PCR amplification comprising
said DNA, polymerase, deoxyribonucleotide triphosphates, a forward AHASL-gene-specific
primer, a reverse AHASL-gene-specific primer, and a mutant-allele-specific primer comprising a
first nucleotide sequence with a 5' end and a 3' end, wherein said first nucleotide sequence is
capable of annealing to the complement of nucleotides 3 to 23 of SEQ ID NO: 12, and wherein
the 3'-end nucleotide of said first nucleotide sequence is cytidine and said cytidine is at the
position as illustrated in Figure 4 that corresponds to the site of the G-to-A point mutation that
gives rise to the S653(At)N substitution in an AHASL protein;

(c) using said DNA as a template for a second PCR amplification comprising
said DNA, polymerase, deoxyribonucleotide triphosphates, said forward AHASL-gene-specific
primer, said reverse AHASL-gene-specific primer, and a wild-type-allele-specific primer
comprising a second nucleotide sequence with a 5' end and a 3' end, wherein said second
nucleotide sequence is capable of annealing to the complement of nucleotides 4 to 23 of SEQ ID
NO: 10, wherein the 3'-end nucleotide of said second nucleotide sequence is guanosine and said
guanosine is at the position as illustrated in Figure 4 that corresponds to the site of the G-to-A
point mutation that gives rise to the S653(At)N substitution in an AHASL protein, and wherein
said wild-type-allele-specific primer comprises the nucleotide sequence set forth in SEQ ID NO:
4[[.]]; and

(d) detecting the products of said first and said second PCR amplifications;

wherein said wild-type-allele-specific primer and said mutant-allele-specific primer are capable of annealing to a region of an *AHASL* gene that is nested between the annealing sites of said forward and reverse *AHASL*-gene-specific primers.

23. (Original) The method of claim 15, wherein said *AHASL* gene is *AHASL1D*.

24. (Previously presented) A method for analysis of a wheat *AHASL* gene, said method comprising the steps of:

(a) obtaining genomic DNA from a wheat plant;
(b) using said DNA as a template for a first PCR amplification comprising said DNA, polymerase, deoxyribonucleotide triphosphates, a forward *AHASL*-gene-specific primer, a reverse *AHASL*-gene-specific primer, and a mutant-allele-specific primer comprising a first nucleotide sequence with a 5' end and a 3' end, wherein said first nucleotide sequence is capable of annealing to the complement of nucleotides 3 to 23 of SEQ ID NO: 12, and wherein the 3'-end nucleotide of said first nucleotide sequence is cytidine;

(c) using said DNA as a template for a second PCR amplification comprising said DNA, polymerase, deoxyribonucleotide triphosphates, said forward *AHASL*-gene-specific primer, said reverse *AHASL*-gene-specific primer, and a wild-type-allele-specific primer comprising a second nucleotide sequence with a 5' end and a 3' end, wherein said second nucleotide sequence is capable of annealing to the complement of nucleotides 4 to 23 of SEQ ID NO: 10, and wherein the 3'-end nucleotide of said second nucleotide sequence is guanosine; and

(d) detecting the products of said first and said second PCR amplifications;
wherein said wild-type-allele-specific primer and said mutant-allele-specific primer are capable of annealing to a region of an *AHASL* gene that is nested between the annealing sites of said forward and reverse *AHASL*-gene-specific primers, wherein said *AHASL* gene is *AHASL1D*, and wherein said forward *AHASL*-gene-specific primer has the sequence set forth in SEQ ID NO: 5 and said reverse *AHASL*-gene-specific primer has the sequence set forth in SEQ ID NO: 7.

Claims 25-28. (Cancelled)

29. (Original) The method of claim 15, wherein said detecting comprises gel electrophoresis and ethidium-bromide staining.

30. (Currently amended) A method for analysis of a wheat *AHASL* gene, said method comprising the steps of:

- (a) obtaining genomic DNA from a wheat plant;
- (b) using said DNA as a template in a pre-amplification comprising said DNA, deoxyribonucleotide triphosphates, polymerase, a forward *AHASL* primer, and a reverse *AHASL* primer, so as to produce pre-amplified DNA;
- (c) using said pre-amplified DNA as a template for a first PCR amplification comprising said pre-amplified DNA, polymerase, deoxyribonucleotide triphosphates, a forward *AHASL*-gene-specific primer, a reverse *AHASL*-gene-specific primer, and a mutant-allele-specific primer comprising a first nucleotide sequence with a 5' end and a 3' end, wherein said first nucleotide sequence is capable of annealing to the complement of nucleotides 3 to 23 of SEQ ID NO: 12, and wherein the 3'-end nucleotide of said first nucleotide sequence is cytidine and said cytidine is at the position as illustrated in Figure 4 that corresponds to the site of the G-to-A point mutation that gives rise to the S653(At)N substitution in an AHASL protein; and
- (d) using said pre-amplified DNA as a template for a second PCR amplification comprising said pre-amplified DNA, polymerase, deoxyribonucleotide triphosphates, said forward *AHASL*-gene-specific primer, said reverse *AHASL*-gene-specific primer, and a wild-type-allele-specific primer comprising a second nucleotide sequence with a 5' end and a 3' end, wherein said second nucleotide sequence is capable of annealing to the complement of nucleotides 4 to 23 of SEQ ID NO: 10, and wherein the 3'-end nucleotide of said second nucleotide sequence is guanosine and said guanosine is at the position as illustrated in Figure 4 that corresponds to the site of the G-to-A point mutation that gives rise to the S653(At)N substitution in an AHASL protein; and
- (e) detecting the products of said first and said second PCR amplifications; wherein said wild-type-allele-specific primer and said mutant-allele-specific primer are

capable of annealing to a region of an *AHASL* gene that is nested between the annealing sites of said forward and reverse *AHASL*-gene-specific primers.

31. (Original) The method of claim 30, wherein said pre-amplified DNA is digested with exonuclease before step (c).

Claims 32-41. (Cancelled)